Journal of Clinical Virology 58 (2013) 559–563

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Preterm prelabor rupture of membranes (PPROM) is not associated with presence of viral genomes in the amniotic fluid

Shubhada Bopegamage[a,*], Marian Kacerovsky[b,c], Vojtech Tambor[c], Ivana Musilova[b,d], Sona Sarmirova[a], Eveline Snelders[e], Arjan S. de Jong[e], Sandor G. Vari[f], Willem J.G. Melchers[e], Jochem M.D. Galama[e]

[a] Enterovirus Laboratory, Medical Faculty, Slovak Medical University, Limbova 12, 83303 Bratislava, Slovak Republic
[b] Department of Obstetrics and Gynecology, Charles University in Prague, Faculty of Medicine in Hradec Kralove, Czech Republic
[c] Biomedical Research Center, University Hospital Hradec Kralove, Czech Republic
[d] Department of Obstetrics and Gynecology, University Hospital Pardubice, Czech Republic
[e] Department of Medical Microbiology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands
[f] International Research and Innovation Management Program, Cedars-Sinai Medical Center, Los Angeles, CA 90048-5502, United States

ARTICLE INFO

Article history:
Received 26 June 2013
Received in revised form 28 August 2013
Accepted 12 September 2013

Keywords:
Pregnancy
Premature rupture of membranes
Amniotic fluid
Viral genomes
Pregnancy outcome

ABSTRACT

Background: The role of viral infections in preterm prelabor rupture of the membranes (PPROM) is not established. Studies on the presence of viral genomes in the amniotic fluid (AF) collected in pregnancies complicated by PPROM show contradictory outcomes.
Objectives: To investigate AF samples of PPROM pregnancies for the presence of viral genomes.
Study design: AF samples from patients with PPROM were collected during a 4-year (2008–2012) observational study. 174 women were included with selection criteria of singleton pregnancy, PPROM, and maternal age of 18 years and above. PCR was used for detection of human cytomegalovirus (HCMV), herpes simplex virus (HSV), parvovirus B19, human adenoviruses (HAdV), enteroviruses (EV) and human parechovirus (HPeV). The selection of these viral targets was based on literature regarding screening of AF for presence of viral genomes.
Results: Only a single sample was positive out of the 174 tested AFs, HCMV DNA was detected.
Conclusions: PPROM is not associated with active viral infections.

1. Background

Preterm prelabor rupture of the membranes (PPROM) is defined as rupture of the fetal membranes with leakage of amniotic fluid (AF) at less than 37 gestational weeks, preceding the onset of uterine activity [1]. PPROM occurs in 2–4% of all pregnancies and represents 30–40% of preterm deliveries, which may have serious consequences for pregnancy outcome, particularly when occurring early in pregnancy [1–4].

Intrauterine infections are a well-known cause of preterm birth [3], but microbial invasion of the amniotic cavity (MIAC) is detected in only 20–50% of PPROM pregnancies, depending on the type of detection techniques applied [5–8]. Intrauterine inflammation is also an important feature of PPROM. Inflammation is reflected by elevated cytokines (interleukin (IL)-6, IL-8 and others) in the AF as well as by presence of neutrophils and other immunoactive cells in the uterine wall, placenta and fetal membranes [9]. The diffuse infiltration of the placenta and fetal membranes is termed histological chorioamnionitis (HCA) [10,11]. Although MIAC correlates in most cases with the presence of HCA, many more cases of HCA (approximately 50%) occur without MIAC being detectable [12]. Non-infectious stimuli, e.g. cell- and tissue damage, may inflict HCA [13], but fastidious infections, which are difficult to detect, may still be involved [5]. Viruses, for example, have been investigated to a lesser extent than bacteria in PPROM pregnancies.

The information about viral invasion of the amniotic cavity and subsequent development of HCA is rather conflicting. Some studies reported absence of viral genomes in second trimester AF samples from low-risk populations [14]. In subsequent studies up to 27% of low-risk second trimester AF samples were positive for adeno-associated viruses (AAV) [15] and from zero to 7% for human adenovirus (HAdV) [14–22]. The consequences of detecting viral genomes in AF for pregnancy outcome are also contradictory. Associations with fetal anomalies/malformations and/or preterm birth were reported by some investigators but were not reproduced by others [16–22]. Remarkably, most studies investigated besides the

* Corresponding author. Tel.: +421 2 593 707 77; fax: +421 2 593706 83.
E-mail address: shubhada.bopegamage@szu.sk (S. Bopegamage).

http://dx.doi.org/10.1016/j.jcv.2013.09.013

established fetal and/or perinatal viral pathogens parvovirus B19, human cytomegalovirus (HCMV) and herpes simplex virus (HSV), also viruses for which a relationship with the pregnancy outcome is uncertain like human papilloma viruses (HPV), respiratory syncytial virus (RSV), influenza virus, Epstein–Barr virus (EBV), human herpes virus-6 (HHV-6), HAdV, AAV and enteroviruses (EV). The significance of detecting genomes of the latter viruses in AF is unclear and remains a subject of debate warranting further investigation [15–22].

A potential role of viruses in PPROM pregnancies has not extensively been investigated. Their presence could provide an explanation for cases where so far no MIAC was detected. The aim of the present investigation was to study well-defined AF samples from PPROM pregnancies for the presence of viral genomes of HCMV, HSV, parvovirus B19, HAdV, EV, HPeV. The selection of most targets was based on existing literature regarding screening of AF for viral genomes. HPeV was added because of its relatedness to EV.

2. Study design

2.1. Patients and samples

Two hundred twenty two pregnant women at gestational ages between 24+0 and 36+6 weeks with PPROM admitted to the Department of Obstetrics and Gynecology, University Hospital Hradec Kralove, Czech Republic between May 2008 and May 2012 have been considered for this study. Selection criteria were singleton pregnancy, PPROM, and maternal age ≥18 years. PPROM was defined as the leakage of AF prior to the onset of labor, which was diagnosed as described before [7,8,12].

Exclusion criteria were clinical chorioamnionitis, diabetes mellitus, hypertension, preeclampsia, signs of fetal growth restriction, the presence of either congenital or chromosomal fetal abnormalities, signs of fetal hypoxia, or significant vaginal bleeding. Moreover, women with ultrasound markers of subclinical infections (intraamniotic and/or fetal inflammatory response) were excluded but not women with potential signs of infection such as small fetal thymus or pulsatile flow pattern in fetal splenic vein. In all pregnancies, the gestational age was established based on first trimester ultrasound evaluation.

Forty-eight women had incomplete data or inadequate samples for histopathology and/or PCR analysis: the remaining 174 women were included into the study.

In the Czech Republic, women with PPROM at less than 34 weeks of gestation are treated with corticosteroids for the induction of lung maturation, tocolytics for 48 h, and antibiotics, whereas no treatment except antibiotics is initiated to delay delivery after 34 weeks. Management of PPROM women in the Czech Republic differs substantially from most clinical guidelines. Details have been described previously and can be found in a National Guideline [7,12,23].

AF sampling, offered to women with PPROM as a part of our local standard protocol was carried out as described previously [7,8,12]. Ultrasound-guided trans-abdominal amniocentesis was performed on admission prior to the administration of corticosteroids, antibiotics, or tocolytics, and approximately 5 ml of AF was aspirated. Upon collection, AF samples were immediately processed as described earlier [7,8,12]. MIAC was determined by PCR for *Ureaplasma* species, *Mycoplasma hominis*, *Chlamydia trachomatis*, or growth of any bacteria in the AF except for coagulase-negative *Staphylococcus epidermidis*, a skin contaminant. HCA was diagnosed by presence of neutrophil infiltration in the chorion-decidua (Grades 3 and 4), the chorionic plate (Grades 3 and 4), the umbilical cord (Grades 1–4), and/or the amnion (Grades 1–4). Funisitis was diagnosed based on the presence of neutrophil infiltration in the umbilical cord (Grades 1–4) [11]. Histopathological samples were reviewed by a single perinatal pathologist (HH), blinded to the clinical status of the women.

Written informed consent was obtained from all subjects. Ninety-three samples from the present study group had been examined in one of our previous reports on the intraamniotic inflammatory response in a subgroup of women with PPROM [12].

2.2. Detection of targeted viral genomes

The viruses selected for real time PCR testing were HCMV, HSV1, HSV2, Parvovirus B19, HAdV, EV and HPeV. Total RNA/DNA was purified from 174 selected AF samples, each spiked with 5 μl of the isolation control Equine Arthritis virus (EAV) and Phocine Herpes virus (PhHV), which served as internal controls. Total nucleic acid (NA) isolation was performed on the MagNA Pure 96 System, using the *MagNA Pure 96 DNA and Viral NA Small Volume Kit* (Roche Diagnostics) and the *Viral NA Plasma SV protocol*. The input and elution volumes were set at 200 μl and 50 μl respectively. A negative control sample (195 μl PBS) was included in each run. RNAs were reverse transcribed by TaqMan Reverse Transcription Reagents kit (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands) in a 50 μl reaction mix containing 20 μl of NA isolate and random hexamers as primers, as per manufacturer's instructions. All reverse transcription (RT) reactions included a negative RT control (PCR grade H_2O instead of template RNA) and a positive RT control (EAV RNA). Real-time PCR mixes (50 μl) consisted of 25 μl of 2× LightCycler®480 Probes Master (Roche), 0.5 μM of each primer and 0.1 μM of each probe, and 5 μl of cDNA or extracted DNA. Real-time PCR was performed on the Roche LightCycler®480 system, with following conditions for all viral targets: 10 min denaturing and hot-start at 95 °C, followed by 50 cycles of 15 s at 95 °C and 15 s at 55 °C, and 20 s at 72 °C. Each real-time reaction included one negative PCR control sample (5 μl of PCR grade H_2O), and one positive control sample (purified plasmid preparations of the respective PCR products).

3. Results

Table 1 shows the demographics and characteristics of the 174 women with PPROM. PPROM pregnancies were divided in categories with HCA and without HCA. Based on histology of placenta and membranes, 109/174 (63%) of the cases had a diagnosis of HCA (data derived from previous studies) [7,8,12].

In only 44/109 (40%) cases with HCA, microbes could be detected in the AF (MIAC) providing an explanation for the intrauterine inflammation. MIAC was detected by culture for aerobic and anaerobic bacteria and PCR for genital mycoplasmas and *Chlamydia trachomatis* as reported previously [7]. The 65 HCA-negative cases could be further subdivided in cases with ($n = 19$), or without evidence for MIAC ($n = 46$). Overall there were 111/174 (64%) patients without MIAC: 65/111 (59%) had the signs of HCA; the remaining 46/111 (41%) were negative for both HCA and MIAC (Table 2).

All 174 AF samples have been tested by a sensitive real-time PCR for presence of HCMV, HSV, parvovirus B19, HAdV, EV and HPeV. Only one sample was positive: CMV-DNA was detected with a load of 5 copies/ml.

4. Discussion

Association between spontaneous preterm delivery and infections has been mainly focused on indigenous bacteria normally present in the vagina, or present due to bacterial vaginosis, and furthermore on genital mycoplasma and/or *Chlamydia trachomatis* infections. Asymptomatic bacteremia has been considered another route of MIAC and evidence therefore was sought in the

Table 1
Demographic and clinical characteristics of women with preterm prelabor rupture of the membranes (PPROM) with respect to the presence of both MIAC and HCA.

	MIAC and HCA present ($n = 44$)	MIAC and HCA either or both absent ($n = 130$)	p-Value
Maternal age (years)	31.3 ± 5.7	31.3 ± 5.5	0.99
Primiparous	13 (30%)	70 (54%)	0.006
Pre-pregnancy body mass index	22.7 (17.0 to 35.7)	22.9 (16.3 to 40.6)	0.60
Gestational age at sampling (days)	30+4 (24+0 to 35+1)	33+3 (24+0 to 36+5)	<0.0001
Gestational age at delivery (days)	31+0 (24+1 to 35+2)	33+5 (24+4 to 36+6)	<0.0001
Smoking during pregnancy	12 (27%)	24 (18%)	0.28
PPROM to amniocentesis interval (h)	8 (2–60)	6 (1–120)	0.31
PPROM to delivery interval (days)	3 (0–10)	2 (0–20)	0.01
Vaginal delivery	30 (68%)	92 (71%)	0.85
Cesarean delivery	14 (32%)	38 (29%)	0.85
Induction of labor	20 (45%)	51 (39%)	0.48
Birthweight (g)	1492 ± 599	2048 ± 593	<0.0001
Apgar score <7; 5 min	7 (16%)	9 (7%)	0.13
Apgar score <7; 10 min	5 (11%)	3 (2%)	0.03
Presence of funisitis	22 (55%)	14 (11%)	<0.0001

HCA: histological chorioamnionitis; MIAC: microbial invasion of the amniotic cavity. The demographic characteristics were compared using either an unpaired t-test or the non-parametric Mann-Whitney U test for continuous variables, and the data are presented as the mean ± standard deviation (SD) and the median (range), respectively. Categorical variables were compared using Fisher's exact test and are presented as percentages (%). The normality of the data was tested using the D'Agostino-Pearson omnibus normality test and the Shapiro-Wilk test. Differences were considered to be statistically significant at $p < 0.05$. The Spearman and partial correlation tests were used to determine the correlation between nucleosome concentrations and gestational age. All p-values were obtained using two-sided tests, and all statistical analyses were performed using the SPSS 19.0 statistical package for Mac OS X (SPSS Inc., Chicago, IL, USA).

Table 2
Histology of membranes and microbiology of AF from 174 women with preterm prelabor rupture of the membranes (PPROM).

Total number of cases tested: $n = 174$			
With HCA $n = 109$ (63%)		Without HCA $n = 65$ (37%)	
With MIAC $n = 44$	Without MIAC $n = 65$	With MIAC $n = 19$	Without MIAC $n = 46$
44/109 (40%)	65/109 (60%)	19/65 (29%)	46/65 (71%)
44/174 (25%)	65/174 (37%)	19/174 (11%)	46/174 (26%)

HCA: histological chorioamnionitis; MIAC: Microbial invasion of the amniotic cavity.

oropharynx, e.g. periodontal infections [24,25]. In spite of the expanded range of detectable microbes a large proportion of cases with intrauterine inflammation and HCA cannot be explained by MIAC.

Viruses could be a cause of inflammation and HCA but studies on intra-amniotic viral infections are sparse and show conflicting outcomes. Antenatal and perinatal infections by HCMV, HIV, parvovirus B19 and HSV are well-established causes of miscarriage, preterm birth, inflammation and postnatal morbidity but the reported data for the other viruses is puzzling.

Table 3 gives an overview of the viral studies in AF from the literature, illustrating the heterogeneity of the studies concerning patient selection, studied viruses and interpretation of the potential role of viruses in pregnancy outcome. Burguette et al. tested 238 AF samples from low-risk pregnancies for AV, AAV, HPV, HSV, and HCMV. In total 44% of samples were positive in the order of occurrence AAV, HPV and HCMV. HAdV and HSV genomes were not detected. The authors reported an association of AAV with PPROM but were cautious about a causal relationship [15]. In a series of studies, all based on molecular assays designed by the Department of Human Genetics, Baylor College Houston, Texas, viral genomes, mainly of HAdV, were detected in 6–8% of AF samples [16–21]. In most of these studies, detection of viral genomes in AF was not associated with an adverse pregnancy outcome [16–18,20], but a significant association (mainly for HAdV genomes) was reported in two of the studies (one prospective and one retrospective in design), a difference that remains unexplained [19,21].

These and additional studies have been reviewed by Gervasi et al. [22]. In their own study of 729 AF samples derived from low-risk pregnancies they detected HHV6, HCMV, parvovirus B19 and EBV in frequency order. Overall 2% of AF samples were positive and there was no association with pregnancy outcome [22]. HAdV genomes were not detected, which contradicts the previously mentioned studies wherein HAdV genomes were the most commonly detected viral genomes in AF [16–21]. A recently published study detected no viral genomes in AF from 13 women with PPROM [26]. In line with the negative studies we did not find viral genomes in a well-defined cohort of 174 PPROM cases, except for a single AF sample that was positive for HCMV DNA at a low concentration of 5 copies/ml of which interpretation is uncertain. The AF sample was furthermore positive for *Ureaplasma urealyticum*. On follow-up the child was healthy. In retrospect, cord blood was tested and showed a fetal inflammatory response with an IL-6 level of 1810 pg/mL and a HCMV DNA load of 100 copies/ml was found which is still of uncertain significance.

Published literature and our own results show a heterogeneous picture suggesting different categories: (i) low-risk pregnancies without viral genomes detected [14], (ii) low-risk (at the time of AF-sampling) pregnancies with positivity ranging between 2 and 41%, a variation that partly depended on the viruses selected for study [15–22], (iii) high-risk pregnancies (PPROM) without detectable viruses or a single positive sample, so far in one study [26] and our present study, and (iv) pregnancies with high suspicion of a viral infection and a correspondingly high percentage (41%) of AF samples that were virus PCR positive [27]. Variations in the viruses investigated and patient selection hampers a serious comparison. A majority of AF samples were drawn in the second trimester and without suspicion of an infection or fetal abnormalities. Particularly for HAdV the results were contradictory. Furthermore, our and some of the other studies observed a poor correlation between amniotic inflammation and detection of virus genomes [16,22].

How can these inconsistencies be explained? Infection of the fetus with HCMV and parvovirus B19 can undisputedly have serious adverse effects: upon vertical transmission, HCMV can cause fetal damage, intra-amniotic inflammation, premature birth and sequelae that develop post-partum [28]. Parvovirus B19 is a cause of adverse pregnancy outcome with, e.g. miscarriage and fetal hydrops [29]. Both viruses can, however, cause asymptomatic infection of the fetus and both can cause latency in the mother from which viral DNA can leak into the circulation [30,31]. Thus, a positive PCR in AF or cord blood may not be sufficient evidence of fetal infection. A formal proof of congenital infection requires probably additional postpartum investigations.

Table 3
An overview of virus studies in the amniotic fluids.

Authors	Source of AF	Gestational age of sampling (weeks)	Relationship of virus in AF to pregnancy/fetal pathology by respective authors	Total samples/patients	DNA viruses									RNA viruses	
					HSV	HHV-6	VZV	HCMV	EBV	HPV	HAdV	AAV	Parvo-B19	RSV	EV
McLean et al. [14]	gAMC with low risk for fetal infection.	12–32	Related to possibility of fetal infection	AF 0/243	0	ND	ND	0	ND	ND	ND	ND	0	ND	ND
Van den Veyver et al. [27]	Women with fetuses at risk for intrauterine viral infection.	15–35	Related to fetal pathology	303[b] AF-95/253	9 3%)[a]	ND	ND	30 10%)	4 (1%)	ND	74 (24%)	ND	8 (3%)	2	22 (7%)
	Controls: gAMC with low risk for fetal infection.	14–22	Unrelated	AF 154	0	ND	ND	1 (1%)	0	ND	3 (2%)	ND	0	0	
Wenstrom et al. [16]	Women with unexplained abortion within 30 days after gAMC.	Second trimester Exact week NA	Unrelated to pregnancy loss	62	1 (2%)	ND	ND	0	0	ND	4 (6%)	ND	0	0	0
	Controls: gAMC with low risk for fetal infection.	Second trimester Exact week NA	Unrelated to pregnancy loss	60	0	ND	ND	3 (5%)	0	ND	5 (8%)	ND	1 (2%)	0	0
Burguete et al. [15]	gAMC.	14–25	No conclusive pathological deduction	As per virus	0	ND	ND	32/183 (18%)	ND	25/208 (27%)	ND	64/238 (27%)	ND	ND	ND
Baschat et al. [17]	gAMC without structural and chromosomal abnormalities.	18 ± 2 (mean ± SD)	Viral genomes maybe present in normal sonography	686	0	ND	ND	5 (1%)	2 (<1%)	ND	37 (6%)	ND	0	1 (<1%)	2 (<1%)
Baschat et al. [18]	gAMC with low risk for fetal infection yielding normal karyotype.	15–27	Related to fetal abnormalities	1090	ND	ND	ND	15 (1%)	2 (<1%)	ND	64 (<1%)	ND	2 (<1%)	2 (<1%)	7 (<1%)
Reddy et al. [19]	gAMC yielding normal karyotype.	19 ± 3 and 20 ± 5 (mean ± SD)	Related to fetal abnormalities	423[c]	0	ND	ND	0	0	ND	(77%)	ND	(5%)	0	(12%)
Miller et al. [20]	gAMC with low risk for fetal infection and normal fetal anatomy and karyotype.	15–23	Viral infection not related to pregnancy outcome	686[d]	ND	ND	ND	4 (10%)	1 (3%)	ND	35 (88%)	ND	0	1 (3%)	2 (5%)
Adams et al. [21]	AMC for karyotyping and viral PCR testing for history or ultrasound based indication.	16–28	Viral infections related to few fetal abnormalities	1191	ND	ND	ND	8 (<1%)	2 (<1%)	ND	59 (5%)	ND	1 (<1%)	2 (<1%)	5 (<1%)
Gervasi et al. [22]	Second trimester AMC for clinical indications.	16–20	Not associated with pregnancy outcome		0	7 (1%)	0	6 (<1%)	2 (<1%)	ND	0	ND	2 (<1%)	ND	0
Naresh and Simhan [26]	PPROM.	24–34	Unrelated to PPROM	13	0	ND	ND	0	ND	0	0	0	0	ND	0

Abbreviations viruses: AAV-2: adeno-associated virus-2; EV: enteroviruses; HAdV: human adenoviruses; HCMV: human cytomegalovirus; HHV: human herpes virus; HPV: human papilloma viruses; HSV: herpes simplex virus; Parvo-B19: parvovirus-B19; RSV: respiratory syncytial virus; VZV: varicella zoster. *Other abbreviations*: AF: amniotic fluid; AMC: amniocentesis; gAMC: genetic amniocentesis; NA: not available; ND: not done; %: percentage.

[a] All presented percentages are rounded to nearest decimal.

[b] Van den Veyver et al. [26] show % PCR positives calculated as postives (shown in table) of 303 patients samples. Total 253 AF of which 95 were positive.

[c] Reddy et al. [19] did not give actual numbers only percentages.

[d] Miller et al. [20] have calculated the % of positive amniotic fluids for only 1 virus ($n = 37$).

Further consideration that a positive PCR can be the result of viral latency in cells, either in the form of episomes or after integration into the host genome, has explicitly been discussed for HHV-6 [22], but it holds also for most of the other viruses studied in AF: for AAV, as was already suggested by Bruguette et al. [15], for EBV which is latently present as episomes in B-lymphocytes [32], and for HAdV for which latency has been reported in T-lymphocytes [33]. Latency has even been reported for EV in peripheral blood mononuclear cells [34]. Consequently, the presence of viral genomes in AF may be the by-product of a physiological cell turn over and of pathological conditions as placental insufficiency and/or inflammation [31,35]. Even more trivial explanations as method of sample collection and handling of material or the primers that are selected for virus detection may explain incongruent outcomes [32]. Of course, the presence of viral genomes in AF may also point to an active infection but that has to be proven by post-natal investigation.

We conclude that in most cases PPROM is not associated with presence of viral genomes and that HCA without detectable MIAC is not explained by an active viral infection. Our hypothesis that detection of viral genomes in AF can reflect latency without any clinical consequence for the fetus requires additional study. This is highly warranted, because it may fundamentally change the interpretation of future AF studies.

Funding

Ministry of Health of the Czech Republic (NT 14104-3/2013) received by MK. Norwegian financial support mechanism. Mechanism EEA and Slovak Government – (SK0082) received by SB.

Competing interest

None.

Ethical approval

This study was approved by the Institutional Review Board committee (March 19, 2008; No. 200804 SO1P), and informed consent was received from all participants.

Acknowledgements

We thank the Cedars-Senai Medical Center International Research and Innovation Management Program and the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association).

References

[1] Mercer BM. Preterm premature rupture of the membranes. Obstet Gynecol 2003;101:178–93.
[2] Caughey AB, Robinson JN, Norwitz ER. Contemporary diagnosis and management of preterm premature rupture of membranes. Rev Obstet Gynecol 2008;1:11–22.
[3] Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med 2000;342:1500–7.
[4] Van der Ham DP, Vijgen SMC, Nijhuis JG, van Beek JJ, Opmeer BC, Mulder ALM, et al. Induction of labor versus expectant management in women with preterm prelabor rupture of membranes between 34 and 37 weeks: a randomized controlled trial. PLoS Med 2012;9:e1001208.
[5] DiGiulio DG. Diversity of microbes in amniotic fluid. Semin Fetal Neonatal Med 2012;17:2–11.
[6] Jacobsson B, Mattsby-Baltzer I, Andersch B, Bokström H, Holst RM, Nikolaitchouk N, et al. Microbial invasion and cytokine response in amniotic fluid in a Swedish population of women with preterm prelabor rupture of membranes. Acta Obstet Gynecol Scand 2003;82:423–31.
[7] Kacerovsky M, Musilova I, Khatibi A, Skogstrand K, Hougaard DM, Tambor V, et al. Intraamniotic inflammatory response to bacteria: analysis of multiple amniotic fluid proteins in women with preterm prelabor rupture of membranes. J Matern Fetal Neonatal Med 2012;25:2014–9.
[8] Kacerovsky M, Pliskova L, Bolehovska R, Skogstrand K, Hougaard DM, Tsiartas P, et al. The impact of the microbial load of genital mycoplasmas and gestational age on the intensity of intraamniotic inflammation. Am J Obstet Gynecol 2012;206:341–8.
[9] Menon R, Taylor RN, Fortunato SJ. Chorioamnionitis a complex pathophysiologic syndrome. Placenta 2010;31:113–20.
[10] Redline RW, Faye-Petersen O, Heller D, Qureshi F, Savell V, Vogler C, et al. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. Pediatr Dev Pathol 2003;6:435–48.
[11] Salafia CM, Weigl C, Silberman L. The prevalence and distribution of acute placental inflammation in uncomplicated term pregnancies. Obstet Gynecol 1989;73:383–9.
[12] Cobo T, Kacerovsky M, Palacio M, Hornychova H, Hougaard DM, Skogstrand K, et al. Intra-amniotic inflammatory response in subgroups of women with preterm prelabor rupture of the membranes. PLoS ONE 2012;7:e41164.
[13] Bianchi ME, Manfredi AA. Dangers in and out. Science 2009;323:1683–4.
[14] McLean LK, Chehab FF, Goldberg JD. Detection of viral deoxyribonucleic acid in the amniotic fluid of low-risk pregnancies by polymerase chain reaction. Am J Obstet Gynecol 1995;173:1282–6.
[15] Burguete T, Rabreau M, Fontanges-Darriet M, Roset E, Hager HD, Koppel A, et al. Evidence for infection of the human embryo with adeno-associated virus in pregnancy. Hum Reprod 1999;14:2396–401.
[16] Wenstrom KD, Andrews WW, Bowles NE, Towbin JA, Hauth JC, Goldenberg RL. Intrauterine viral infection at the time of second trimester genetic amniocentesis. Obstet Gynecol 1998;92:420–4.
[17] Baschat AA, Towbin J, Bowles NE, Harman CR, Weiner CP. Prevalance of viral DNA in amniotic fluid of low-risk pregnancies in the second trimester. J Matern Fetal Neonatal Med 2003;13:381–4.
[18] Baschat AA, Towbin J, Bowles NE, Harman CR, Weiner CP. Is adenovirus a fetal pathogen? Am J Obstet Gynecol 2003;189:758–63.
[19] Reddy UM, Baschat AA, Zlatnik MG, Towbin JA, Harman CR, Weiner CP. Detection of viral deoxyribonucleic acid in amniotic fluid: association with fetal malformation and pregnancy abnormalities. Fetal Diagn Ther 2005;20:203–7.
[20] Miller J, Harman C, Weiner C, Baschat AA. Perinatal outcomes after second trimester detection of amniotic fluid viral genome in asymptomatic patients. J Perinat Med 2009;37:140–3.
[21] Adams LL, Gungor S, Turan S, Kopelman JN, Harman CR, Baschat AA. When are amniotic fluid viral PCR studies indicated in prenatal diagnosis? Prenat Diagn 2012;32:88–93.
[22] Gervasi M, Romero R, Bracalente G, Chaiworapongsa T, Erez O, Dong Z, et al. Viral invasion of the amniotic cavity (VIAC) in the midtrimester of pregnancy. J Matern Fetal Neonatal Med 2012;25:2002–13.
[23] The Czech Society of Obstetrics and Gynecology. Clinical guidelines in obstetrics; 2007. Available in Czech language at: http://www.perinatologie.cz/dokumenty
[24] Agarwal V, Hirsch E. Intrauterine infection and preterm labor. Semin Fetal Neonatal Med 2012;17:12–9.
[25] Behrman RE, Butler AS. Preterm birth: causes, consequences, and prevention. Committee on Understanding Premature Birth and Assuring Healthy Outcomes; 2007. ISBN: 0-309-65898-5, 790 pages, 6–9, http://www.nap.edu/catalog/11622.html
[26] Naresh A, Simhan H. Absence of virus in women with PPROM: a case series. J Reprod Immunol 2012;96:79–83.
[27] Van den Veyver IB, Ni J, Bowles N, Carpenter Jr RJ, Weiner CP, Yankowitz J, et al. Detection of intrauterine viral infection using the polymerase chain reaction. Mol Genet Metab 1998;63:85–95.
[28] Gaytant MA, Steegers EA, Semmekrot BA, Merkus HM, Galama JM. Congenital cytomegalovirus infection: review of the epidemiology and outcome. Obstet Gynecol Surv 2002;57:245–56.
[29] Young NS, Brown KE. Parvovirus B19. N Engl J Med 2004;350:586–97.
[30] Boom R, Sol CJA, Schuurman T, van Breda A, Weel JFL, Beld M, et al. Human cytomegalovirus DNA in plasma and serum specimens of renal transplant recipients is highly fragmented. Clin Microbiol 2002;40:4105–13.
[31] Lo YM, Chiu RW. Prenatal diagnosis: progress through plasma nucleic acids. Nat Rev Genet 2007;8:71–7.
[32] Stevens SJ, Pronk I, Middeldorp JM. Toward standardization of Epstein–Barr virus DNA load monitoring: unfractionated whole blood as preferred clinical specimen. J Clin Microbiol 2001;39:1211–6.
[33] McNees AL, Mahr JA, Ornelles D, Gooding LR. Postinternalization inhibition of adenovirus gene expression and infectious virus production in human T-cell lines. J Virol 2004;78:6955–66.
[34] Schulte BM, Bakkers J, Lanke KH, Melchers WJ, Westerlaken C, Allebes W, et al. Detection of enterovirus RNA in peripheral blood mononuclear cells of type 1 diabetic patients beyond the stage of acute infection. Viral Immunol 2010;23:99–104.
[35] Hromadnikova I. Extracellular nucleic acids in maternal circulation as potential biomarkers for placental insufficiency. DNA Cell Biol 2012;31:1221–32.